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Various ketogenic diets can differently support brain resistance against experimentally evoked seizures and seizure-induced elemental anomalies of hippocampal formation

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ABSTRACT

In this paper the influence of two different ketogenic diets (KDs) on the seizure-evoked elemental anomalies of hippocampal formation was examined. To achieve this purpose normal and pilocarpine treated rats previously fed with one of the two high fat and carbohydrate restricted diets were compared with animals on standard laboratory diet. The ketogenic ratios of the examined KDs were equal to 5:1 (KD1) and 9:1 (KD2).

KD1 and standard diet fed animals presented similar patterns of seizure-evoked elemental changes in hippocampal formation. Also the analysis of behavioral data recorded after pilocarpine injection did not show any significant differences in intensity and duration of seizures between KD1 and standard diet fed animals.

Higher ketogenic ratio KD2 introduced in the normal hippocampal formation prolonged changes in the accumulation of P, K, Zn and Ca. Despite this, both the intensity and duration of seizures were significantly reduced in rats fed with KD2 which suggests that its saving action on the nerve tissue may protect brain from seizure propagation. Also seizure-evoked elemental anomalies in KD2 animals were different than those observed for rats both on KD1 and standard diets. The comparison of seizure experiencing and normal rats on KD2, did not show any statistically significant differences in elemental composition of CA1 and H hippocampal areas whilst in CA3 area only Zn level changed as a result of seizures. DG was the area mostly affected by seizures in KD2 fed rats but areal densities of all examined elements increased in this hippocampal region.

1. Introduction

Epilepsy is one of the most common neurological conditions that affect people of all ages. In approximately one third from 50 million people suffering from epilepsy currently available drugs are ineffective in controlling seizures [1,2]. What is more, pharmacoresistant epilepsy is associated with five-fold higher mortality rate when compared to general population [3]. Therefore, there is a great need for the development of new therapeutic strategies and one of them can be ketogenic diet (KD) [4].

The KD is a high-fat, low carbohydrate, normocaloric diet that mimics the metabolic state of fasting [5]. Because of low carbohydrate intake during the treatment with KD body tissues are forced to catabolize fats as their primary source of energy. In turn, metabolism of fats leads to the production of ketone bodies which become alternative energy substrates to glucose [6].

For around hundred years KD has been utilized for the management of refractory seizures. It is mostly used to treat pediatric epilepsies but gives satisfactory results also in adolescents and adults [7–9].

Lefevre and Aronson reviewed and synthesized the existing literature concerning the efficacy of KD diet in reducing seizure frequency for children with refractory epilepsy. The results based on retrospective and prospective studies showed that complete cessation of all seizures occurred in 16% of children treated with KD. Thirty two percent of patients had a greater than 90% reduction in seizures, whilst in fifty six percent of them greater than 50% reduction in seizures occurred [10]. The evidence shows, moreover, that KD may also improve long-term outcome in epileptic children beyond the period of the diet [11,12].

Although KD has been used in medical practice for many decades [13,14], the mechanisms of its therapeutic action are still rather obscure [15,16]. Therefore further study is necessary for better understanding of these mechanisms. In turn this, should improve clinical

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utilization and effectiveness of KD as well as limit its side effects.

A few different formulas of KD are used in clinical practice [17]. In the most typical KDs the mass ratio of fats to proteins plus carbohydrates (ketogenic ratio) is equal to 3:1 or 4:1 [18]. The KDs with higher ketogenic ratios are usually more efficient in the seizure control but may be worse tolerate by the patients [19]. Our previous investigations showed, moreover, that elemental and biochemical changes occurring in the hippocampal formation after KD depended on its composition [20–22]. This suggests that also the mechanisms of anticonvulsive action of different KDs may not be the same.

In this paper we tried to verify how KD modified elemental anomalies occurring in hippocampal formation as a result of pilocarpine evoked seizures. To achieve this, rats previously fed with two different KDs were compared with animals on standard laboratory diet. The ketogenic ratios of the examined KDs were equal to 5:1 (KD1) and 9:1 (KD2). Besides the content of main nutrients, the KDs 1 and 2 differed in the proportions of unsaturated and saturated fats as well as concentrations of some from the analyzed elements.

For highly spatially resolved elemental analysis of hippocampal formations synchrotron X-ray fluorescence microscopy was applied [23,24]. The areal densities of elements with the atomic number from 15 to 34 were determined for sectors 1 and 3 of the Ammon's horn (CA1 and CA3 respectively), dentate gyrus (DG) and hillus of DG (H).

2. Materials and methods

2.1. Animals

Male Wistar rats came from an animal colony of the Department of Neuroanatomy (Institute of Zoology, Jagiellonian University). All animal-use procedures were carried out there and were approved by the Bioethical Commission of the Jagiellonian University in accordance with international standards. The animals were maintained under conditions of controlled temperature (20 ± 2 °C) and illumination (12-h light:12-h dark cycle).

On the 30th day of postnatal development the animals were divided into the six groups which were fed either with one of the KDs (K1, K2, K1SE and K2SE groups) or with standard laboratory diet (N and NSE groups). The characteristic of the examined groups was presented in Table 1. Moreover, in Table 2 the contents of main nutrients, fatty acids and selected elements in both KDs and standard laboratory diet were compared.

2.2. Seizure induction and behavioral observations

On the 60th day of postnatal development, the rats from NSE, K1SE and K2SE groups received a single i.p. injection of pilocarpine (250 mg/kg, Sigma P6503) in order to induce seizures. Additionally, they were injected with scopolamine methyl bromide (1 mg/kg, Sigma S8502) 30 min prior to pilocarpine to reduce its peripheral effects. These procedures were done between 9 and 10 a.m. to avoid circadian changes in seizure vulnerability.

Table 1
The characteristic of examined animal groups.

Experimental group	KD1[a]	KD2	Standard diet	Pilocarpine[b]	Perfusion[c]
N (n[d] = 7)			+		+
NSE (n = 6)			+	+	+
K1 (n = 6)	+				+
K1SE (n = 6)	+			+	+
K2 (n = 6)		+			+
K2SE (n = 7)		+		+	+

[a] Ketogenic diets 1 and 2 (KD1 and KD2) were introduced to rats on the 30th day of postnatal life.
[b] Pilocarpine was injected to rats on the 60th day of postnatal life.
[c] Perfusion with physiological saline solution was done on the 60th day of animals life, 6 h after pilocarpine injection in case of animals with pilocarpine evoked seizures.
[d] The number of rats in the experimental group.

Table 2
The content of main nutrient (in [%]), fatty acids (in [g/kg]) and selected elements (in [mg/g]) in the dry mass of diets.

Nutrient	KD1	KD2	Standard diet
Lipids	75	79	5
Carbohydrates	5	1	63
Proteins	9	8	25
Others	11	12	7
SFAs[a]	348	329	–[b]
MUFAs[a]	277	330	–
PUFAs[a]	115	86	–
P	4100	5700	4100
S	160	–	160
K	2200	7900	2200
Ca	7800	7900	7800
Fe	88	138	88
Cu	5.9	11	5.9
Zn	32	51	32
Se	0.41	0.10	0.41

[a] Saturated (SFAs), monounsaturated (MUFAs) and polyunsaturated (PUFAs) fatty acids.
[b] Lack of information.

After pilocarpine injection, the rats were continuously observed during the 6-h period. Motor symptoms were rated on the six-point scale used in our previous studies [25]. Additionally, the observations provided the data on general parameters of *status epilepticus* such as the time when the maximal seizures occurred and the total time of seizure activity within the observation period.

To evaluate the influence of KDs on the progress of pilocarpine evoked seizures the behavioral parameters recorded during the observation period for K1SE and K2SE groups were compared with those measured for seizure experiencing rats on the standard diet.

The time when the seizures of maximal intensity occurred did not differ significantly between the three examined groups. The comparison of intensity of maximal seizures as well as the duration of seizure activity within the observation period gave different results. One can see in Fig. 1 where the dispersions of these behavioral parameters recorded for K1SE, K2SE and NSE groups were shown as box-and-whisker plots.

As one can notice from Fig. 1 both intensity of maximal seizures and the duration of seizure activity within the observation period were significantly lower in animals fed with higher ketogenic ratio KD. Such differences were not found for KD1 fed rats in case of which behavioral parameters did not differ significantly from those recorded for animals on standard diet and presented significantly higher values comparing to rats from K2SE group.

2.3. Sample preparation

Six hours after injection of pilocarpine all animals were perfused with physiological saline solution of high analytical purity. The brains were quickly excised, frozen and cut using a cryomicrotome into 12 μm thick sections. The slices of the dorsal part of the hippocampus were mounted on the ultrapure (without trace elements), ultrathin (4 μm) and transparent for X-rays Ultralene® foil and freeze-dried.

2.4. Measurements

X-ray fluorescence microscopy was used for the topographic and quantitative analysis of elements such as: P, S, K, Ca, Fe, Cu, Zn and Se. The measurements were carried out at the FLUO beamline of ANKA Synchrotron Facility in frame of the proposals A2013-022-004153 and A2014-024-006393. The energy of the exciting beam was equal to 16 keV and the beam was focused with polycapillary optics to a spot